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Real-time measurements using an automated staining and fluorescence detection microfluidic-based system for cell-based assays.

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This paper reports on an improved feedback control system to ensure optimal industrial cell production. Industrial production of cells has a need for greater process control, despite of the standard temperature, pH, oxygen concentration and number of dead and live cells controls [1]. Monitoring cells health through a vast range of fluorescence cell-based assays can greatly improve the feedback control and thereby ensure optimal cell production. Thus an automated system capable of staining the cell population and performing automated image analysis on a multi-use microfluidic chip performing hourly analysis for a period of 6 – 12 months would be ideal.

The present system relies on the use of miniaturized peristaltic pumps and multi-channel valves with similar working principles as in [2] – Figure 1. Our system differs from previous work in the implementation of computer controlled commercial stepper motors (MP042NB340 Rev P2, from GCM A/S, Denmark), the use of re-usable and durable in-house designed fluidic interconnections (Figure 2) for interfacing thermally bonded micro-milled PMMA microfluidic chips (Figure 3), and the use of highly resistant pump tubing (RCT-ISMT-1-TPE, from RCT, Germany. All in all, these modifications make such a system more robust, flexible, durable and automated.

Large scale bioreactors can easily achieve cell concentrations of 10^{10} cells/ml, which severely difficult automated image analysis, as cells are too closely packed. To deal with this challenge the developed system was devised to perform simultaneously sample staining and dilutions of 1:10 up to 1:500, by combining channels flow rates of 1 to 350 µl/min (± 5 %).

The system workflow consists in dragging a cell sample from a bioreactor, staining and diluting the cell sample in a serpentine mixing structure (Figure 2), followed by fluorescence imaging of the stained cells in a shallow chamber (0.1 mm), in order to ensure that all cells are on the same plane. Once the cell sample has been imaged (Figure 4. a)), 0.1 M NaOH is flushed through the system for 5 min (Figure 4. b)). The washing buffer has a dual effect since it kills the cells, and also washes away cells adsorbed to the walls, since it is flushed at a high flow rate – 150 µl/min.

Validation of the current system was done by staining yeast cells (saccharomyces cerevisiae) with Vitabright-48 and PI (provided by ChemoMetec A/S, Denmark). The use of both dyes enables a fast method of assaying apoptosis as no washing or incubation steps are necessary [3]. Fluorescence images were obtained using a fluorescence microscope (Axio Imager.M1, Carl Zeiss, Germany). Figure 4 a) illustrates that the cell dilution/staining provides a S/N high enough (5x magnification) to enable the determination of the initial cell concentration. Furthermore Figure 4 b) illustrates that the washing step is sufficient to remove all cells and therefore enables the chip to be used multiple times.

The ability to automate cell staining and image analysis opens a wide repertoire of assays for improving feedback controls used in bioreactors.

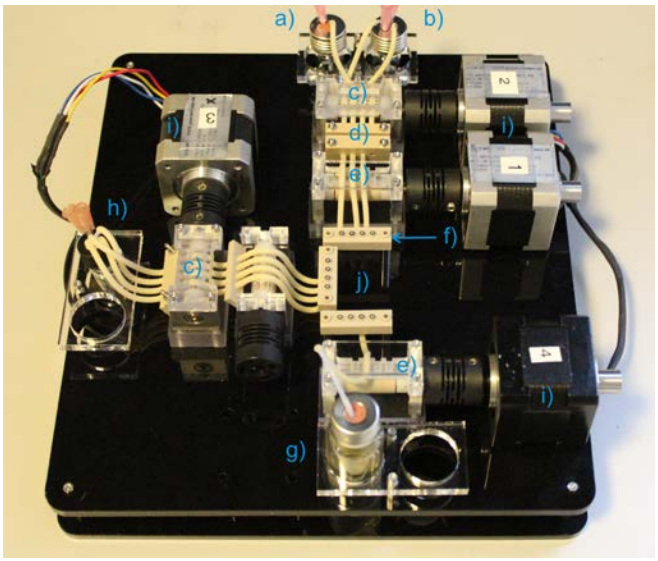

Figure 1. Automated staining/dilution and fluorescence detection platform. a) Sample reservoir/connection to bioreactor; b) Washing buffer(0.1 M NaOH); c) Peristaltic pump; d) Stoppers; e) Valve; f) Microfluidic connectors; g) Waste reservoir; h) Fluorescence dye reservoir; i) Stepper Motors; j) Place to insert the microfluidic chip.

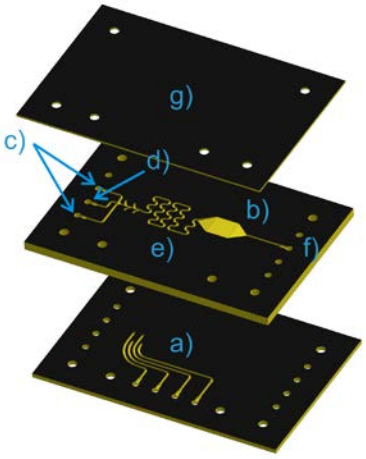

Figure 3. a) Staining/dilution microfluidic channels (cross section 0.5 x 0.5 mm^2); b) Fluorescence imaging chamber (w: 5 mm, l: 5 mm, d: 0.1 mm); c) Washing buffer inlets; d) Sample inlet; e) Serpentine mixing channel; f) outlet; g) lid (0.5 mm thick)

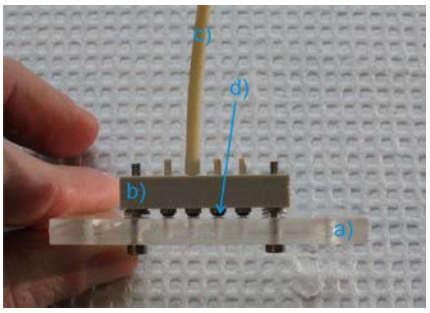

Figure 2. In-house designed microfluidic connector (PEEK). a) PMMA microfluidic chip (side-view); b) PEEK connector; c) Tubing; d) Black rubber o-ring.

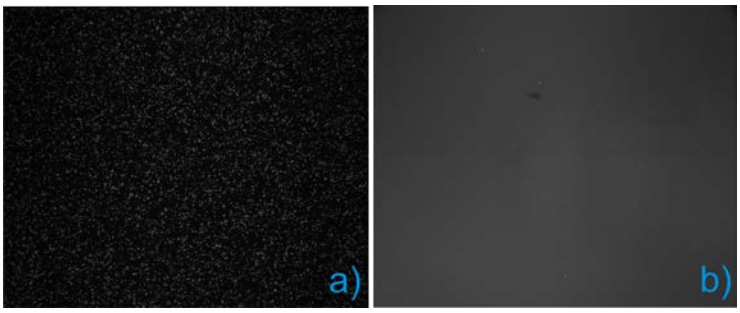

Figure 4. Fluorescence images - 5x magnification. a) Yeast (saccharomyces cerevisiae) stained with Vitabright48. b) Imaging chamber after purging with 0.1 M NaOH.

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